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Cortical and subcortical chemical pathology in Alzheimer's disease as assessed by multislice proton magnetic resonance spectroscopic imaging

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Article abstract—*Background:* Multislice proton magnetic resonance spectroscopic imaging (1H-MRSI) permits the simultaneous acquisition of *N*-acetylaspartate (NA), choline (Cho), creatine/phosphocreatine (Cre), and lactate (Lac) signal intensities from four 15-mm slices divided into 0.84-ml single-volume elements. NA is inferred to be a neuron-specific molecule, whereas Cho mainly reflects glycerophosphocholine and phosphocholine, compounds involved in phospholipid metabolism. *Objective:* To assess whether 1H-MRSI could detect a regional pattern of cortical and subcortical involvement in the brain of Alzheimer's disease (AD) patients. *Methods:* 1H-MRSI was performed in 15 patients with probable AD and 15 age-matched healthy controls. Regions of interest (ROIs) were selected from frontal (FC), temporal (TC), parietal (PC), occipital, and insular cortices, subcortical white matter (WM), and thalamus. *Results:* In AD patients, we found a significant reduction of NA/Cre in the FC, TC, and PC and a significant reduction of Cho/Cre in the WM. *Conclusions:* This 1H-MRSI study of AD patients shows a regional pattern of neuronal damage in the associative cortices, as revealed by significant reduction of NA/Cre in the FC, TC, and PC, and regional derangement of phospholipid metabolism, as revealed by significant reduction of Cho/Cre in the WM.

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Neuronal loss and abnormalities of membrane phospholipids[1-4] are implicated in the pathophysiology of Alzheimer's disease (AD). Magnetic resonance imaging (MRI) can depict neuronal loss in the form of brain atrophy; however, the degree of neuronal loss can be underestimated because of reactive gliosis.

Proton magnetic resonance spectroscopy (1H-MRS) provides, in a noninvasive way, insight into in vivo brain metabolism for a number of chemicals, and it has been successfully applied to the study of central nervous system (CNS) disorders.[5-8] The principal metabolite signals detected by 1H-MRS at long echo-time (TE) arise from *N*-acetyl–containing compounds with *N*-acetylaspartate (NA) as the prominent contributor, choline-containing compounds (Cho), creatine/phosphocreatine (Cre), and lactate (Lac). NA is inferred to be a neuron-specific molecule because it is absent in both mature glial cell cultures and tumors of glial cell origin,[9-12] and immunofluorescence studies have shown that fluorescent anti-NA antibodies co-localize with antibodies against neuron-specific proteins.[13,14] The Cho peak reflects total brain choline stores,[15] with major contributions from glycerophosphocholine and phosphocholine.[16] The Cre peak reflects the total amount of phosphocreatine and creatine, which are involved in energy metabolism.[15] Lac signal rises whenever the glycolytic rate exceeds the capacity of oxidative metabolism or, possibly, when the capacity for exporting lactate to the bloodstream is impaired.[17]

In AD, 1H-MRS studies of postmortem brain samples have shown a reduction of NA in the visual, temporal, frontal, and parahippocampal regions,[18] in the posterior temporoparietal cortex,[19] and in the superior and middle frontal cortex and superior temporal cortex[20]; a reduction of Cre was found as well.[19] In vivo 1H-MRS studies of AD also have shown a decrease of NA in the occipital gray matter,[21,22] in the frontal regions of the brain,[23] in the parietal and occipital cortices,[24] and in the mesial gray matter at the level of the centrum semiovale.[25] Cho was found to be either unchanged[22,23] or increased[25] in the posterior section of the centrum semiovale.

Previous in vivo 1H-MRS studies were based on single-volume[21-24] or multi-voxel[25] techniques. During the past few years, spectroscopy technology has evolved to multislice 1H-MRS imaging (1H-MRSI). The latter technique permits the simultaneous acquisition of NA, Cho, Cre, and Lac signal intensities from four 15-mm slices divided into 0.84-ml single-

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volume elements[26] and enables mapping of the spatial extent of metabolic abnormalities with improved resolution. The acquired data can be displayed in a tomographic format, thus making multislice 1H-MRSI suitable for the study of diffuse CNS disorders and of large and heterogeneous CNS lesions.[27-32]

In the present study, we used multislice 1H-MRSI to measure in vivo the metabolite signal intensities of NA, Cho, Cre, and Lac in the brain of patients with AD. Our purpose was to establish whether multislice 1H-MRSI could detect a regional pattern of cortical and subcortical involvement in the brain of AD patients.

Subjects and methods. We studied 15 patients with probable AD, aged 55 to 77 years (mean ± SD, 68 ± 8.0 years), and 15 age- and sex-matched healthy volunteers as control subjects. All 15 AD patients met National Institute of Neurological Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINDS-ADRDA) criteria for probable AD.[33] The mean Mini Mental State Examination (MMSE) score[34] was 14.6 ± 7.8 with a range of 5 to 28, and the mean disease duration was 3.8 years ± 0.7. AD patients were free of other concomitant diseases. The protocol was approved by the Institutional Review Board, and all subjects or their legal guardians gave written informed consent for the study.

Due to the relatively long duration of the 1H-MRSI and MRI study (60 minutes), mainly patients with a mild to moderate grade of involvement (13 out of 15) could complete the examination and were included in the present study.

Cerebral atrophy rating scale. Magnetic resonance imaging (MRI) studies of all patients and controls were interpreted by a senior neuroradiologist (N.J.P.), without prior knowledge of the clinical or spectroscopic findings, to rate brain atrophy and leukomalacia. The presence or absence of atrophy was determined by examining the width of the cortical sulci, the size of the sylvian fissures, and the ventricular volume, and a score of overall brain atrophy was assigned. Leukomalacia lesions were evaluated from the characteristic white matter high signal changes on the T2-weighted images. The number and the extent of these abnormalities were measured to determine the severity of involvement. A four-grade scale was used to rate both atrophy and leukomalacia, which were characterized as absent, mild, moderate, or marked.

1H-MRSI studies. The 1H-MRSI studies were performed on a 1.5-tesla MR imager equipped with self-shielded gradients (GE Medical Systems, Milwaukee, WI) using a previously described data acquisition procedure and a standard quadrature imaging head coil.[26] Images were obtained at an angle of approximately +20 degrees from the orbitomeatal line to depict the maximum number of regions of interest (ROIs) throughout the brain. Phase encoding procedures were used to obtain a 32 × 32 array of spectra from voxels with a nominal volume of 0.84 ml (7.5 mm × 7.5 mm × 15 mm) within the selected slices. The 1H-MRSI data acquisition comprised a multiple-slice spin echo slice selection with a repetition time (TR) of 2,200 msec and an echo time (TE) of 272 msec. Outer-volume signal saturation was used to suppress signals arising from the skull marrow and surface tissues. Four 15-mm-thick slices with 2.5-mm interslice spacing were acquired.

The raw data from each slice consist of a set of 804 phase-encoded full spin echoes. The phase encodings sample a circular region of the k-space centered at the origin. Each echo consists of 256 complex points sampled 1 msec apart (sweep width = 1 kHz). The echoes were apodized by multiplying the nth point by the factor:

$$\begin{cases} \sin(np/128) & \text{for } n < 64 \\ 1 & \text{for } 64 \le n \le 192 \\ \sin([256-n]p/128) & \text{for } n > 192. \end{cases}$$

The echoes were zero filled to 512 points and Fourier transformed to the frequency domain, the k-space at each point in the frequency domain was zero filled to form a 32 × 32 square matrix (with k = 0 at the center), and the k-space domain was apodized by multiplying spectrum l,m ($-15 < l,m < 16$) by $\sin([1 - 0.5]\pi/33) \sin([m - 0.5]\pi/33)$ before Fourier transforming to yield a 32 × 32 array of spectra.

To generate metabolite signal intensities images, the following procedure was used: first the magnitude for each spectrum was computed, then an automated peak-picking program was used to identify the Cho, Cre, NA, and Lac peaks, then the peak identification was reviewed and manually changed for any in which the automatic procedure had failed, and spectra from voxels contaminated by fat were zeroed out. After the peaks in all the spectra were properly identified, the metabolite values were determined for each pixel by summing the magnitude spectrum over a frequency band of width 6 Hz (0.1 ppm) centered at each peak. The magnitude values were integrated to produce four 32 × 32 arrays showing spatial variation of the strength of each of the signals in each of the selected slices.

Conventional 3-mm T1- and T2-weighted MRIs, collected immediately after the 1H-MRSI acquisition, were used to identify the ROIs, encompassing defined neuroanatomic structures within the brain. ROIs containing an integral number of 0.84-ml spectroscopic voxels were drawn on the T1-weighted and T2-weighted MRIs and transferred to the identical location on all 1H-MRSI to obtain metabolite signal intensities in these locations. ROIs were selected from the frontal (FC), temporal (TC), parietal (PC), occipital (OC), and insular cortices (IC), subcortical white matter (WM), and thalamus (TH), according to the following criteria: (a) the ROIs should fit entirely within the same neuroanatomic structure in at least four of the five 3-mm MRIs that correspond to the 15-mm 1H-MRSI, (b) voxels showing poor spectral resolution (less than half-height separation of Cho and Cre signals) or residual water signal were excluded. The ROIs were chosen by two of us (A.B. and N.L.), who had no knowledge of the diagnosis. For each ROI, mean metabolite signal intensity ratios (NA/Cho, NA/Cre, Cho/Cre) were calculated for the patients and normal subjects. Whenever possible, bilateral ROIs were obtained for side-to-side comparisons.

Statistical analysis. Metabolic differences of patients versus controls for each ROI for each ratio were examined with a 2 × 2 analysis of variance (ANOVA) with the following factors: hemisphere (left or right) and diagnosis (patients with AD versus controls). Post hoc analysis was performed by means of the Tukey honest significance difference test. Bonferroni correction for multiple regions was

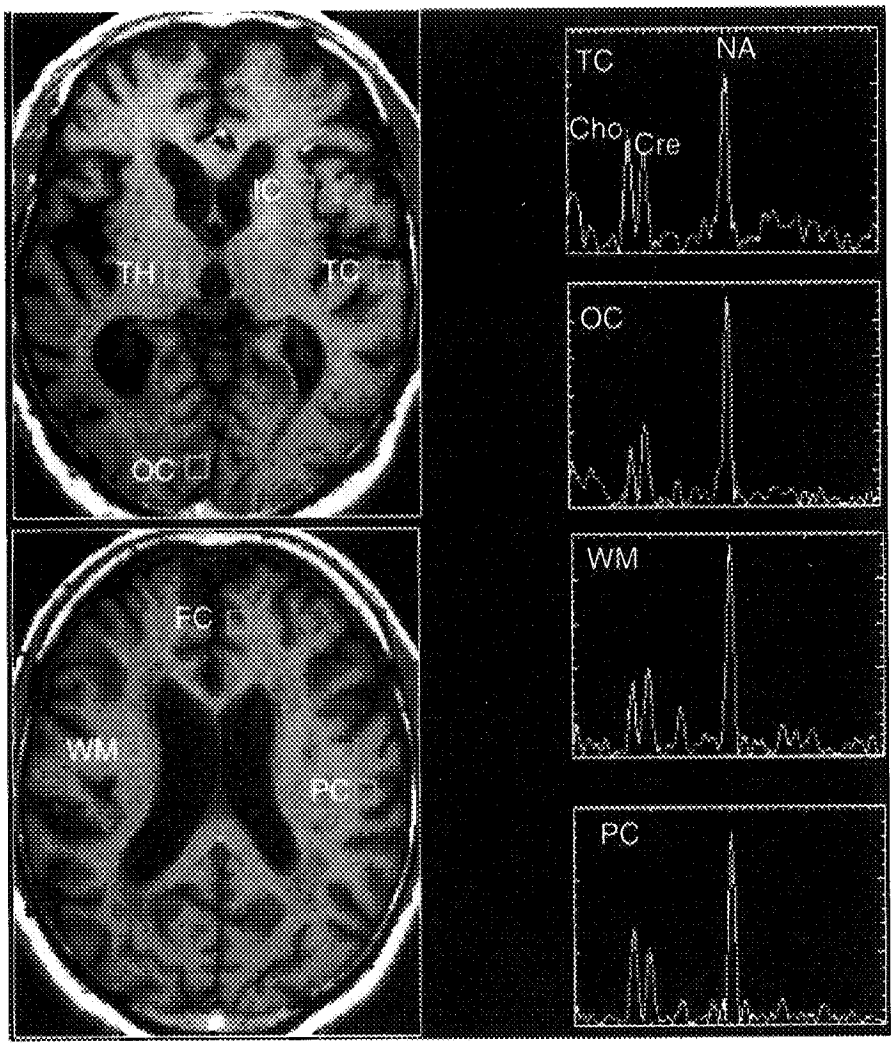

Figure 1. Location of the regions of interest (ROIs) and representative spectra in one AD patient. ROIs are frontal cortex (FC), temporal cortex (TC), parietal cortex (PC), occipital cortex (OC), insular cortex (IC), subcortical white matter (WM), and thalamus (TH). Cho = choline; Cre = creatine; NA = N-acetylaspartate.

applied. Spearman rank correlational analysis was also used to test for correlations between metabolite ratios and brain atrophy and MMSE.

Results. Figure 1 shows the location of the ROIs and representative spectra from one AD patient.

Figures 2 and 3 show MRI and the corresponding metabolite images of NA, Cho, and Cre signal intensities of one healthy volunteer and one patient.

Figure 4 shows means and SDs of the three metabolite ratios (NA/Cre, NA/Cho, and Cho/Cre) in patients and normal controls. AD patients had lower mean NA/Cre than normal controls in the FC (mean ± SD of AD patients versus controls: 2.16 ± 0.22; 2.43 ± 0.35), TC (2.10 ± 0.17; 2.40 ± 0.33), and PC (2.06 ± 0.38; 2.46 ± 0.46), and lower Cho/Cre in the WM (1.17 ± 0.16; 1.35 ± 0.14). ANOVA revealed a significant effect of diagnosis for NA/Cre in the FC (F 10.3; *df* 1,46), TC (12.7; 1,33), and PC (9.3; 1,40), and Cho/Cre in the WM (15.2; 1,38). Post hoc analysis showed that AD patients had significantly (Bonferroni-corrected) reduced NA/Cre in the FC ($p < 0.01$), TC ($p < 0.007$), and PC ($p < 0.03$), and significantly reduced Cho/Cre in the WM ($p < 0.003$). No significant differences between AD patients and controls were found in any other ROI for any other metabolite signal intensities ratio.

In the control group, brain atrophy was absent in 12 subjects and mild in 3; mild leukomalacia was found in only 1 subject. In the AD group, brain atrophy was absent in 6 patients, mild in 4, and moderate in 5; leukomalacia was absent in 8 patients, mild in 3, and moderate in three. The quality of the images in 1 patient was not sufficient to assess white matter abnormalities.

There were no significant correlations between 1H-MRSI findings and either brain atrophy or MMSE scores.

No Lac signals were found in the patients or normal subjects. This finding is consistent with the normal intracerebral Lac concentration of approximately 0.5 μmol/g, which is close to or below the detection limit of the method used.

Discussion. The present multislice 1H-MRSI study of AD patients showed a regional pattern of neuronal damage in the associative cortices, as revealed by a significant reduction in NA/Cre in the FC, TC, and PC, and regional derangement of phospholipid metabolism as revealed by a significant reduction in Cho/Cre in the WM. The absence of significant differences in the Cho/Cre in FC, TC, and PC between patients and controls leads to the conclusion that a reduction of the NA/Cre ratio is due to a reduction of NA. Furthermore, in AD, Cre was significantly reduced in one in vitro 1H-MRS study,[19] and reduced, though nonsignificantly, in an in vivo quan-

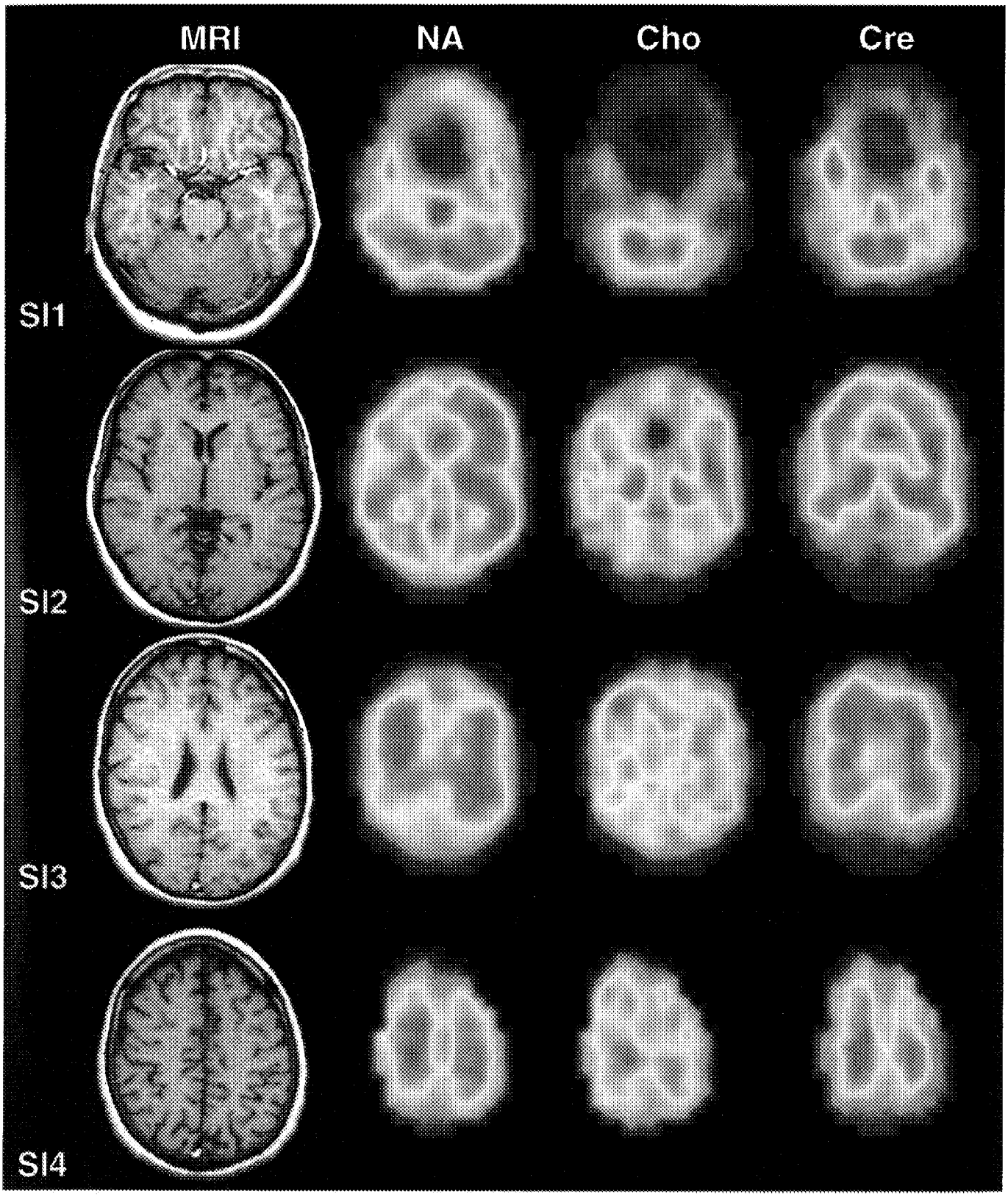

Figure 2. MRI and 1H-MRSI of a control. The MRI slice is 3 mm thick and corresponds to the center of the 15-mm thick 1H-MRSI slice. Cho = choline; Cre = creatine; NA = N-acetylaspartate. 1H-MRSI data are displayed using a color scale that depicts the strongest signal integral with red and the weakest with dark blue. The 1H-MRSI data are displayed at their nominal voxel in-plane resolution (7.5 mm × 7.5 mm). Color images are scaled to the highest value of each metabolite signal intensity for each 1H-MRSI slice, so that the pattern of regional distribution of metabolite signal intensities within the same slice can be compared between subjects, although color intensity from the same anatomic location cannot be compared between subjects.

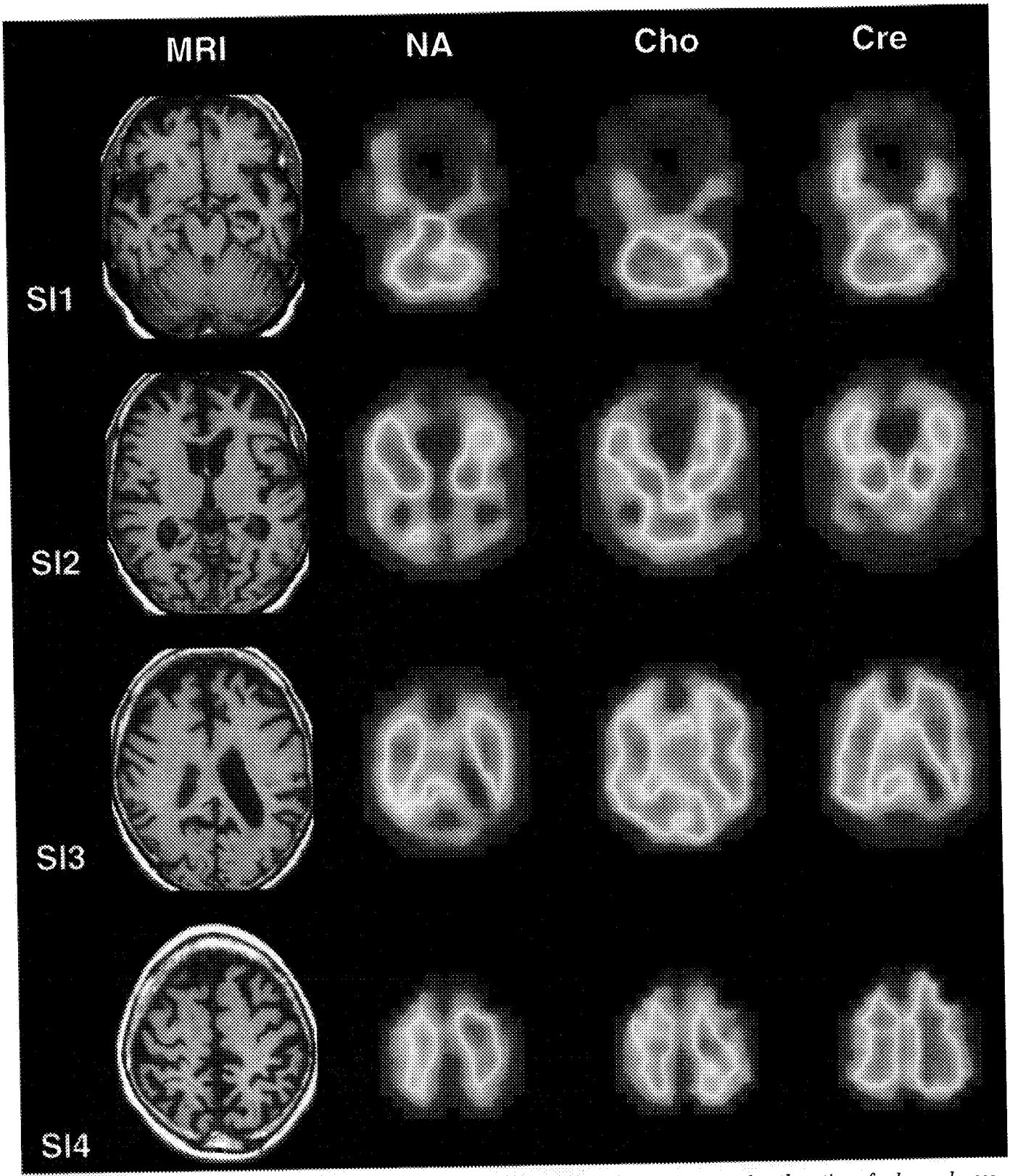

Figure 3. MRI and 1H-MRSI of a patient with Alzheimer's disease. For abbreviations and explanation of color scale, see figure 2.

titative localized 1H-MRS study.[22] These findings[19,22] suggest that the reduction of NA/Cre and Cho/Cre found in our patients is not likely due to a relative increase of Cre.

A number of positron emission tomography (PET) studies, recently reviewed by Jagust,[35] have indicated that in the early stages of AD, metabolic decrements are focal and predominantly involve the parietal and temporal lobes, whereas the metabolic involvement becomes more generalized with disease

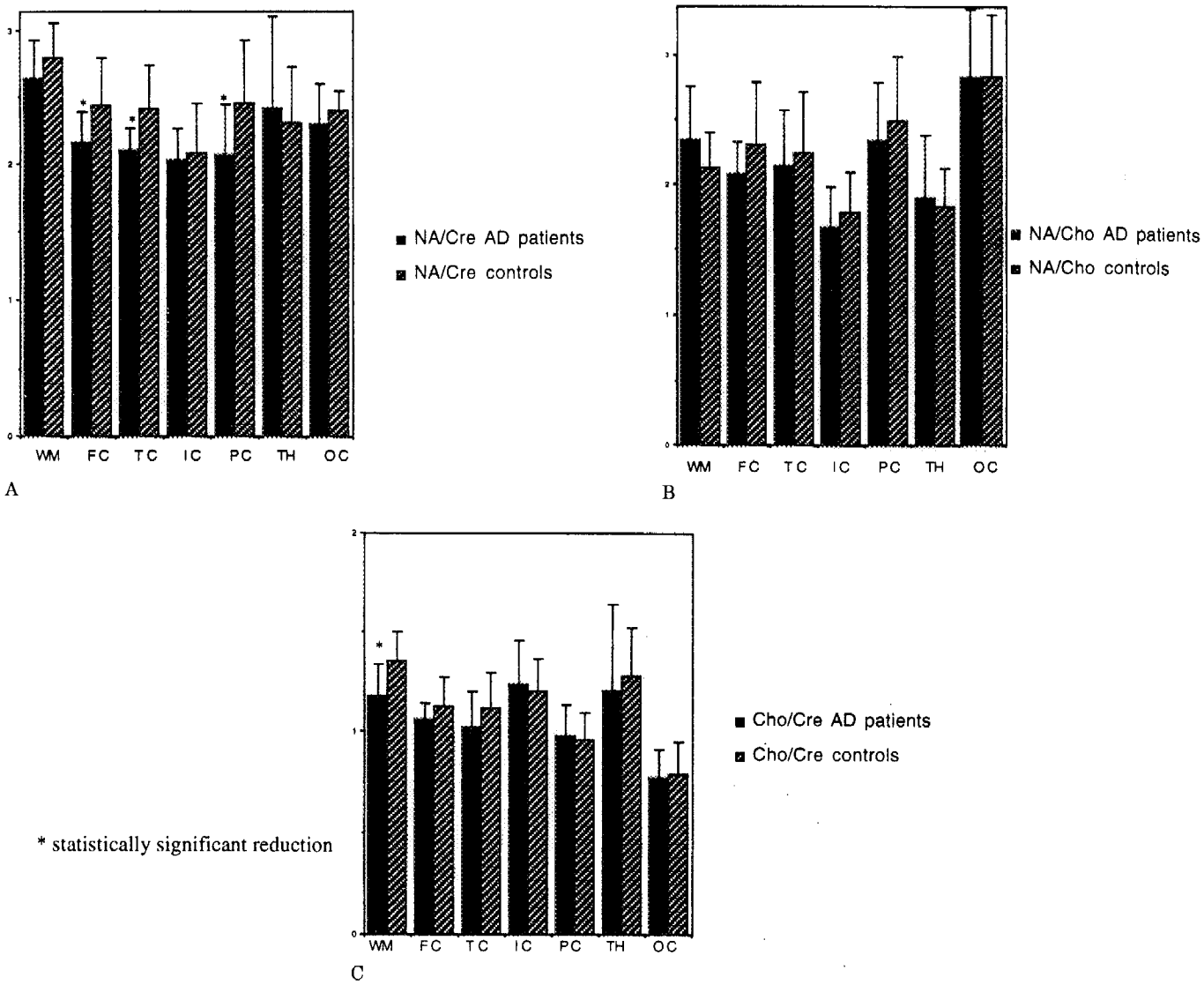

Figure 4. Regional variations (mean + SD) of NA/Cre (A), NA/Cho (B), and Cho/Cre (C) in AD patients and controls. Regions of interest are frontal cortex (FC), temporal cortex (TC), parietal cortex (PC), occipital cortex (OC), insular cortex (IC), subcortical white matter (WM), and thalamus (TH).

progression. Consistent with PET findings, neuropathologic studies[36-38] suggest that though some neuropathologic changes in AD are widespread throughout the neocortex, the association areas of the parietal, temporal, and frontal regions are disproportionally affected. The results of the present multislice 1H-MRSI study are consistent with previous neuropathologic and PET findings in AD. Indeed, neuronal loss, as revealed by significant reduction of NA/Cre, was present only in FC, TC, and PC.

A number of in vivo 1H-MRS studies have shown a reduction of NA in parietal, temporal, frontal, and occipital cortices in AD,[21-25] and in some of these investigations the reduction of NA has been measured with quantitative methods.[21-23] These studies, however, could not accurately differentiate between white and gray matter due to the relatively large size of the volumes studied (ranging from 2.2 to 15 mL). In addition, by limiting the metabolic analysis to one or, at the most, a few brain areas, these investigations yielded information that may not be representative of the actual disease process, with its predilection for specific structures. The multislice 1H-MRSI technique, used in our study, allowed the selection of smaller volumes (0.84 mL) and the simultaneous assessment of the spatial extent of metabolic abnormalities in AD in a more comprehensive way. The results of the present 1H-MRSI study are in agreement with previous 1H-MRS data on frontal[23] and parietal areas,[24] while they are not consistent with previous 1H-MRS data on occipital gray matter of AD patients.[21,22,25] The latter discrepancy might be due to differences in patient selection, with our patients being mainly in a mild to moderate stage of the disease as reflected by the limited degree of atrophy and white matter abnormalities. How-

ever, methodologic differences between studies might also play a role; choice and volume of ROIs are likely to be more accurate in the present study, while quantitative methods, as well as short TE sequences, are likely to be more sensitive in previous 1H-MRS studies.

Despite the advantages of multislice 1H-MRSI to study the brain of AD patients, some caveats remain. Due to the extensive folding of the thin cortical gray matter ribbon with white matter, cortical spectroscopic voxels, even of the order of 0.84 mL, may contain a mixture of both tissue types. Furthermore, because of cerebral atrophy, some spectroscopic voxels could be contaminated with cerebrospinal fluid (CSF). However, even if a markedly greater amount of CSF were present in the selected ROIs of the patients, all metabolite signals would be equally decreased without affecting the metabolite ratios. Finally, we could not select an adequate number of voxels from the hippocampus due to the angle used and to the often poor quality of spectra from the hippocampus.

We anticipate that correcting 1H-MRSI data for tissue composition, as assessed by MRI segmentation technique, could improve the accuracy of 1H-MRSI to differentiate between gray matter and white matter abnormalities. MacKay et al.[39] have combined 1H-MRSI and MRI segmentation to study AD, in a multi-voxel 1H-MRSI study aimed at ROIs located at the level of the centrum semiovale. They found that metabolic changes, as measured by 1H-MRSI, were independent of tissue type differences, as measured by MRI segmentation.

We did not obtain metabolite relaxation times (T1 and T2) because of the prohibitively long examination times required. We used a relatively long TR (TR/[T1 of NA] > 1) and long TE (TE/[T2 of NA] < 1) for data acquisition. T1 and T2 relaxation time differences of 1H-MRSI metabolites could potentially cause differences in signal intensities. Our primary observables were signal intensity ratios. The ratio of two signal intensities (S_a/S_b) is influenced by the relaxation times (T1 and T2) of the metabolite signals according to the following equation which may be derived from first principles based on knowledge of the pulse sequence.

$$S_a/S_b = C_a/C_b * C_{T1} * C_{T2}$$

where

$$C_{T1} = (1 - \exp\{-TR/T1_a\})/(1 - \exp\{-TR/T1_b\})$$

$$C_{T2} = (\exp\{-TE/T2_a\})/(\exp\{-TE/T2_b\})$$

and C_a/C_b is the true concentration ratio. C_{T1} and C_{T2} may be viewed as correction factors that express how much the relaxation times affect the measured signal intensity ratios. Estimates of T1 and T2 from single-volume 1H-MRS studies in the literature permit one to calculate the approximate values of C_{T1} and C_{T2}. The relaxation time values of Kamada et al.[40] for normal brain using our TR and TE values provide the following estimates:

Ratio	C_{T1}	C_{T2}
NA/Cre	0.95	1.33
Cho/Cre	1.03	1.43
NA/Cho	0.92	0.93

If we make the assumption that metabolite signal relaxation times are regionally invariant as is tacitly done above, then the correction factors for metabolite signal ratios from different locations (i.e. NA_{TH}/NA_{WM}) would be 1.00 in all cases. The analysis provided is limited in that it ignores the possibility of T1 and T2 alterations associated with region or with disease. It also ignores uncertainties that are inherent in the measurement of the relaxation times. To our knowledge, there is no evidence that 1H-MRSI metabolites have abnormal T1 in AD patients. Furthermore, Christiansen et al.[23] in a single-voxel study from frontal cortex, found that the T2 relaxation time for NA was longer in AD patients relative to controls. Assuming that the T2 relaxation time for NA is not regionally different, the true NA level in AD patients, if anything, may be overestimated.

In the present study, we found a significant decrease of Cho/Cre ratio in the WM of AD patients. We are inclined to relate this finding to a reduction of Cho signal intensity, rather than to an increase of Cre signal intensity because such an increase has never been described in AD, while in one in vitro study, a reduction of Cre was found.[19] The decrease of Cho/Cre is consistent with biochemical data showing reduction of myelin components,[41] a decrease of cerebrosides in the white matter but not in the cortex,[42] and an increase of lipid peroxidation products as an indicator of myelin alterations in AD brains.[43] In previous in vivo 1H-MRS studies, AD was associated with either unchanged[22,23] or increased[25] Cho in the posterior section of the centrum semiovale. Meyerhoff et al.[25] suggested that the increase of Cho supports the theory of increased phospholipid turnover or decreased glycerophosphocholine degradation, or both, in AD.[2,3] The Cho peak reflects both membrane precursors (phosphocholine) and degradation products (glycerophosphocholine) as well as other contributors, possibly from mobile phosphatidylcholine.[15,16] The exact nature of these changes may be different in different brain regions and at different stages of the illness, and it is likely that changes in Cho/Cre, both as a decrease or as an increase, indicate alteration in phospholipid metabolism. Constans et al.[44] found that in the white matter of AD patients the increase of Cho correlated positively with the presence of white matter signal hyperintensities. We suggest that differences between our study and the previously mentioned ones[25,44] might reflect differences both in the choice of ROIs and in disease progression.

We did not find a significant decrease of NA/Cre in

the WM of AD patients, although there was a trend to a decrease of NA/Cre. This could reflect the limitations of estimating metabolite changes by ratios when a simultaneous decrease of two or more metabolites occurs. Indeed, in one quantitative in vivo 1H-MRS study of AD patients,[22] all three metabolites were decreased, although only the decrease of NA was statistically significant. However, the present finding of a relative preservation of NA/Cre with respect to Cho/Cre in the WM, is consistent with the neuropathologic findings suggesting that in AD the myelin compartment is more vulnerable to mild ischemia than the axonal.[45]

The lack of significant correlations between 1H-MRSI findings and brain atrophy and MMSE could be due to the characteristics of our AD patients. They showed a limited degree of brain atrophy (9 out of 13 had mild to moderate brain atrophy) and a MMSE score lower than 10 in only 5 patients. Conversely, the lack of correlation may be due to the characteristics of the methods, where 1H-MRSI offers neurochemical information, MRI shows atrophy, and MMSE reflects the mental status of AD patients. These correlations need to be further investigated.

Shonk et al.[21] assessed the diagnostic specificity and sensitivity of single-volume 1H-MRS, located in the occipital lobes, in a large number of AD patients. They found that reduced levels of NA and even more increased levels of *myo*-inositol characterize AD and concluded that 1H-MRS enables identification of mild to moderate AD with a specificity and sensitivity that suggest clinical utility. The results of the present 1H-MRSI study, although not aimed at measuring specificity and sensitivity, are consistent with such a conclusion. This technique, by mapping the spatial extent of metabolic abnormalities with improved resolution throughout most of the brain, may provide useful adjunctive information in the diagnosis of AD and eventually become a new tool to evaluate the progression of the disorder and the effect of therapy.

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